Data Evaluation Record on the Acute Toxicity of NAK 4455 (Transfluthrin) Technical to Algae, *Scenedesmus subspicatus*

PMRA Submission Number {.....} EPA MRID Number 49617843

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	436376
	OECD Data Point	{.....}
	EPA MRID	49617843
	EPA Guideline	850.4500

Test material: **NAK 4455 (Transfluthrin) Technical** **Purity:** 95%
Common name
Chemical name: IUPAC (2,3,5,6-tetrafluorophenyl)-methyl-(IR-trans)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
CAS name: Not Reported
CAS No.: Not Reported
Synonyms: Not Reported

Primary Reviewer: Moncie V. Wright
Environmental Scientist, CDM/CSS-Dynamac JV
Signature: Moncie V Wright
Date: 2/9/2017

Secondary Reviewer: John Marton, Ph.D.
Environmental Scientist, CDM/CSS-Dynamac JV
Signature:
Date: 2/13/2017

Primary Reviewer: Frank T. Farruggia, Ph.D.
Senior Scientist, EPA/OCSPP/OPP/EFED/ERB1
Date: 9/11/2017 2017.09.11 15:38:50 -04'00'

Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA}
Date: {.....}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	129140	

Date Evaluation Completed: 11-09-2017

CITATION: F Heimbach. 1987. Growth inhibition of green algae (*Scenedesmus subspicatus*) caused by NAK 4455 (techn.). Study conducted by Bayer AG, Leverkusen, Germany. Study report no.: HBF/AL 38. Study sponsored by Bayer CropScience. Study completed August 20, 1987.

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study,

on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Scenedesmus subspicatus* (strain SAG 86/81) were exposed to **NAK 4455 (Transfluthrin) Technical** at nominal concentrations of 0 (negative and solvent control) and 0.1 mg ai/L under static conditions.

The % growth inhibition in the treated algal culture as compared to the control was 6%. There were no treatment-related effects on any endpoint tested in this study. Additionally, there were no compound related phytotoxic effects.

The overall NOAEC and IC_{50} values are 0.1 and >0.1 mg ai/L, respectively.

The water solubility of the test material was reported to be 0.057 mg/L; therefore, the limit concentration selected by the study author was acceptable according to EPA guidance.

This study **is scientifically sound** and is classified as **supplemental (quantitative).** The study was downgraded because the reviewer could not determine if the single exposure concentration was prepared taking into account the % purity of the test material (95%), the water solubility of the test material was reported to be 0.057 mg/L, and the analytical verification of the test material was not performed.

Results Synopsis

Test Organism: *Scenedesmus subspicatus* (strain SAG 86/81)
Test Type (Flow-through, Static, Static Renewal): Static

Yield, Area under the curve, and Growth rate
IC_{05}: N/A 95% C.I.: N/A
*IC_{50}: >0.057 mg ai/L 95% C.I.: N/A
*NOAEC: 0.057 mg ai/L
Probit Slope: N/A

*no analytical verification of tested concentration was provided. Therefore the solubility in water was used for the endpoint, the actual exposure is likely higher than this because of the use of acetone as a solvent.

Endpoint(s) Effected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with ISO Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition Test, 1984), and/or the OECD Guideline No. 201, "Alga, Growth Inhibition Test" (1984). The reviewer assessed the study methods according to U.S. EPA OCSPP 850.4500: *Algal Toxicity* (2012) and OECD 201 (2011), describing similarities and/or differences where they existed. Deviations were noted:

1. Analytical verification of the test material was not performed. OCSPP and OECD guidance recommend that analytical confirmation be performed at a minimum at test initiation and termination.
2. Only 3 replicates were established for the controls and single exposure group. OCSPP guidance recommends a minimum of 4 replicates in order to provide acceptable confidence in the results, whereas a minimum of 6 replicates is recommended under OECD guidance in the case of limit tests.
3. The study author did not report the source of the dilution water, and did not provide information on water quality as recommended by OCSPP guidance. OECD guidance does not address this topic.
4. Random assignment of treatments to test vessels and test vessels to growth chamber positions was not reported. OCSPP and OECD guidance recommend random assignment, and OCSPP guidance states that the absence of random assignment can render a study invalid.

These deviations do not have an impact on the acceptability of this study.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. This study was conducted in compliance with GLP regulations as published by the U.S. EPA (40 CFR Part 160 and 792; 1983) and OECD (1981). A dated Quality Assurance certificate was provided, but a signature was not included.

A. MATERIALS:

1. Test material **NAK 4455 (Transfluthrin) Technical**

Description: Brown liquid

Lot No./Batch No. : Mixed batch 130187

Purity: 95%

Stability of compound under test conditions: Analytical verification of the test material in the solutions tested was not performed.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not reported.

Physicochemical properties of NAK 4455 (Transfluthrin) Technical.

Parameter	Values	Comments

Parameter	Values	Comments
Water solubility at 20°C	Not reported.	
Vapor pressure	Not reported.	
UV absorption	Not reported.	
pKa	Not reported.	
Kow	Not reported.	

2. Test organism:

Name: *Scenedesmus subspicatus*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: SAG 86/81
Source: In-house laboratory culture.
Age of inoculum: (at test initiation)
Method of cultivation: Incubated under 16L:8D at 20°C in autoclaved nutrient solution, with weekly sterile inoculations into fresh nutrient solution.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A range-finding study was conducted with a negative and solvent control and nominal concentrations of 0.01 and 0.1 mg ai/L. The maximum inhibition recorded was 10%, in the lowest test level for the endpoint area under the curve.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	Precultures were inoculated, and 3 days later the algae were used for the test.	

Parameter	Details	Remarks
		Criteria
Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Temperature was similar; specific information regarding the algal medium was not provided. Algae were taken from an exponentially growing preculture.	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system Static/static renewal Renewal rate for static renewal	Static N/A	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Controlled environment cabinet	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass Erlenmeyer flasks 300-mL 100-mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name		

Parameter	Details	Remarks
		Criteria
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	7.74 to 8.14 8.36 to 8.40 $Na_2EDTA \times 2H_2O$ N/A N/A	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes.	
<u>Dilution water</u> source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Not reported. Not determined. N/A Deionized and filtered. Not determined. Not determined. Not determined. Not determined. Not determined.	 *EPA pH: <u>Skeletonema costatum</u> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	The test material (131.6 mg) was dissolved in acetone (100 mL), then an aliquot was diluted in deionized filtered water and agitated for 4 hours with a magnetic stirrer. From this stock solution, the single test concentration treatments were prepared using 10x concentrated nutrient medium.	

Parameter	Details	Remarks
		Criteria
Aeration or agitation	The flasks were agitated at 3 revolutions per minute.	
Initial cells density	1 x 10^4 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricormutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates Control: Solvent control: Treatments:	 3 3 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations		

Parameter	Details	Remarks *Criteria*
Nominal: Measured:	0 (negative and solvent controls), and 0.1 mg ai/L N/A- analytical verification not performed	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	Acetone; 0.1 mL/L (0.01%)	
Method and interval of analytical verification	N/A- analytical verification was not performed	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 22.5 to 22.7°C Continuous 8000 lux	 *EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	 $K_2Cr_2O_7$ p.a. 0.18, 0.32, 0.56, 1.0, and 1.8 mg/L	
Other parameters, if any	None to report	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	- Cell density - Area under the curve - Growth rate	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	The cell counts were indirectly determined via photometric measurements of the extinction/turbidity using a 1-ray photometer (WTW MPM 1,500). The cell counts were calculated using the extinction values. The other endpoints were calculated using the acquired cell counts, but the equations were not reported.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	Samples were taken randomly for microscopy to determine if there were atypical cell sizes that might unduly affect the extinction measurements.	
Indicate whether there was an exponential growth in the control	Yes. At test termination, averaged cell density in the negative control was 261 x 10^4 cells/mL.	*EPA requires control cell count at termination to be ∟2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The negative and solvent control and nominal 0.1 mg ai/L treatment group were similar across all endpoints. The maximum inhibition calculated by the reviewer was 6% (for cell density and yield). No compound-related phytotoxic effects were reported.

Table 3: Effect of NAK 4455 (Transfluthrin) Technical on algal growth *Scenedesmus subspicatus*.

Treatment Nominal concentration (mg ai/L)	Initial cell density (x 10^4 cells/mL)	Cell density (x 10^4 cells/mL) at			
		48 hours	72 hours	96 hours	
				cell count	% inhibition[1]
Negative control	1	29	101	261	N/A
Solvent control	1	28	99	261	0
0.1	1	27	102	245	6
Reference chemical (if used)	Not reported				

1 Inhibitions calculated by the reviewer relative to the negative control.

Table 4: Effect of NAK 4455 (Transfluthrin) Technical on algal growth *Scenedesmus subspicatus*.[1]

Treatment Nominal concentration (mg ai/L)	Area under the curve		Growth rate (x 10^4 cells/mL/day)		Cell yield (x 10^4 cells/mL)	
	0-96 hours	% inhibition[2]	0-96 hours	% inhibition[2]	0-96 hours	% inhibition[2]
Negative control	6348	N/A	1.39	N/A	260	N/A
Solvent control	6236	2	1.39	0	260	0
0.1	6104	4	1.38	1	244	6

1 Means calculated by the reviewer.
2 Inhibitions calculated by the reviewer relative to the negative control.

Table 5: Statistical endpoint values.*

Statistical Endpoint	Cell density	Growth rate	Area under the curve
NOAEC or EC_{05} (mg ai/L)	Not determined	0.1	0.1
IC_{50} (mg ai/L)	Not determined	>0.1	>0.1
Reference chemical, if used NOAEC (mg ai/L) IC_{50}/EC_{50} (mg ai/L)	Not determined	 Not reported 1.2	 Not reported 0.44

* Do not use this table, if the study was deemed unacceptable.

B. REPORTED STATISTICS:

Statistical analyses were not conducted.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer assessed the endpoints cell yield, growth rate, and area under the curve using CETIS version 1.8.7.12 statistical software using backend database settings implemented by EFED on 10/20/15. The negative and solvent controls were first evaluated using an equal variance t two-sample test. No significant differences were determined, and the single treatment group was compared to the negative control.

All endpoints were evaluated for normality and homogeneity of variance using Shapiro-Wilk's test and the Variance Ratio F test, respectively. Area under the curve data were normal, but were not homoscedastic, and were analyzed using an unequal variance t two-sample test. Yield and growth rate data were normal and homoscedastic, and were analyzed using an equal variance t two-sample test.

The ICx values could not be calculated due to a lack of effects in this study ≥50%. All toxicity values were reported in terms of the nominal exposure concentration.

Yield, Area under the curve, and Growth rate
IC_{05}: N/A 95% C.I.: N/A
IC_{50}: >0.1 mg ai/L 95% C.I.: N/A
NOAEC: 0.1 mg ai/L
Probit Slope: N/A

D. STUDY DEFICIENCIES:

Analytical verification of the test material in the treated algal media was not performed.

E. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were in complete agreement. There was no toxicity in this study.

The reviewer independently calculated area under the curve, growth rate, and yield using the study author's cell count data and used those data for the reviewer's statistical analyses.

The reviewer could not determine if the single exposure concentration was prepared taking into account the % purity of the test material (95%).

The water solubility of the test material was reported to be 0.057 mg/L.

The in-life phase of the test was conducted from August 3 to August 7, 1987.

F. CONCLUSIONS:

This study **is scientifically sound** and is classified as **supplemental (quantitative)**. There were no treatment-related effects on any endpoint tested in this study. The overall NOAEC and IC_{50} values are 0.1 and >0.1 mg ai/L, respectively.

Yield, Area under the curve, and Growth rate
IC_{05}: N/A 95% C.I.: N/A
IC_{50}: >0.057 mg ai/L 95% C.I.: N/A
NOAEC: 0.057 mg ai/L
Probit Slope: N/A

Endpoint(s) Effected: None

III. REFERENCES:

None provided.

OCSPP 850.4500 Algal Toxicity — **Bayer AG**

Analysis ID:	05-4300-0132	**Endpoint:**	96h AUC	**CETIS Version:**	CETISv1.8.7
Analyzed:	09 Feb-17 13:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-3415-0746	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	03 Aug-87	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Not specified
Ending Date:	07 Aug-87	**Species:**	Scenedesmus subspicatus	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	14.7%	Passes 96h auc

Unequal Variance t Two-Sample Test

Control	vs	C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		0.1	0.761	2.92	936	2	0.2630	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	89304	89304	1	0.58	0.4888	Non-Significant Effect
Error	616128	154032	4			
Total	705432		5			

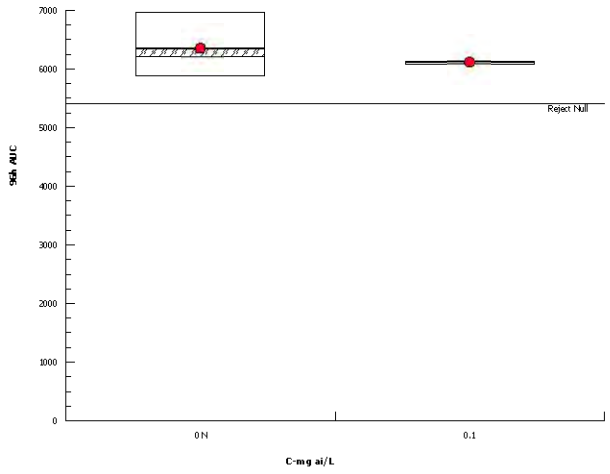
Distributional Tests

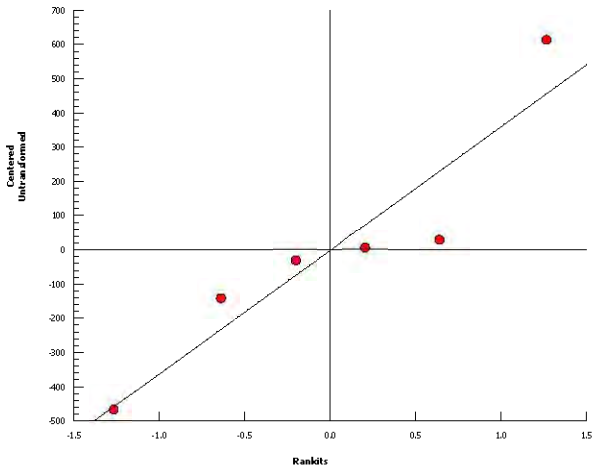
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	337	199	0.0059	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.903	0.43	0.3915	Normal Distribution

96h AUC Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	3	6350	4970	7720	6200	5880	6960	320	8.73%	0.0%
0.1		3	6100	6030	6180	6110	6070	6130	17.4	0.5%	3.84%

Graphics





OCSPP 850.4500 Algal Toxicity — **Bayer AG**

Analysis ID:	12-7447-2159	**Endpoint:**	96h AUC	**CETIS Version:**	CETISv1.8.7
Analyzed:	09 Feb-17 13:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-3415-0746	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	03 Aug-87	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Not specified
Ending Date:	07 Aug-87	**Species:**	Scenedesmus subspicatus	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C <> T	NA	NA	15.1%	Passes 96h auc

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		Solvent Blank	0.325	2.78	958	4	0.7617	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	18816	18816	1	0.105	0.7617	Non-Significant Effect
Error	713760	178440	4			
Total	732576		5			

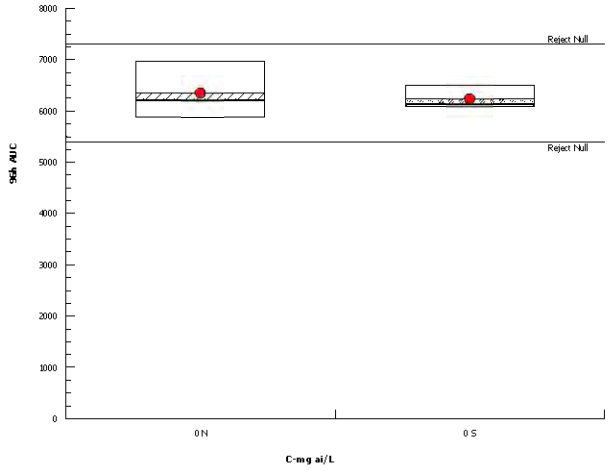
Distributional Tests

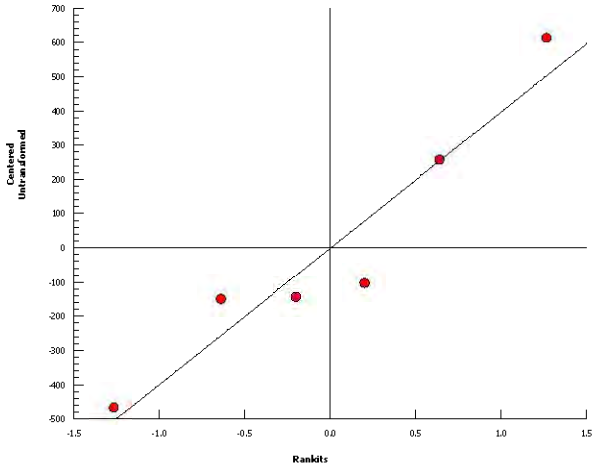
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	6.18	199	0.2787	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.925	0.43	0.5408	Normal Distribution

96h AUC Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	3	6240	5680	6790	6130	6080	6490	129	3.58%	0.0%
0	Negative Control	3	6350	4970	7720	6200	5880	6960	320	8.73%	-1.8%

Graphics





OCSPP 850.4500 Algal Toxicity — **Bayer AG**

Analysis ID:	16-7848-5057	**Endpoint:**	96h Cell Density	**CETIS Version:**	CETISv1.8.7
Analyzed:	09 Feb-17 13:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-3415-0746	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	03 Aug-87	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Not specified
Ending Date:	07 Aug-87	**Species:**	Scenedesmus subspicatus	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	8.58%	Passes 96h cell density

Equal Variance t Two-Sample Test

Control	vs	C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		0.1	1.56	2.13	22.3	4	0.0970	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	400.1667	400.1667	1	2.43	0.1940	Non-Significant Effect
Error	658.6667	164.6667	4			
Total	1058.833		5			

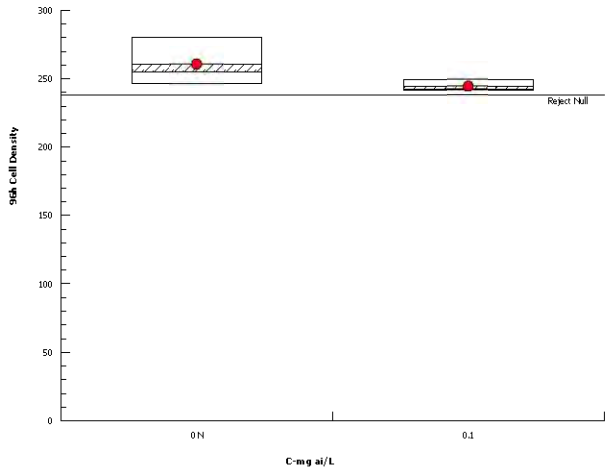
Distributional Tests

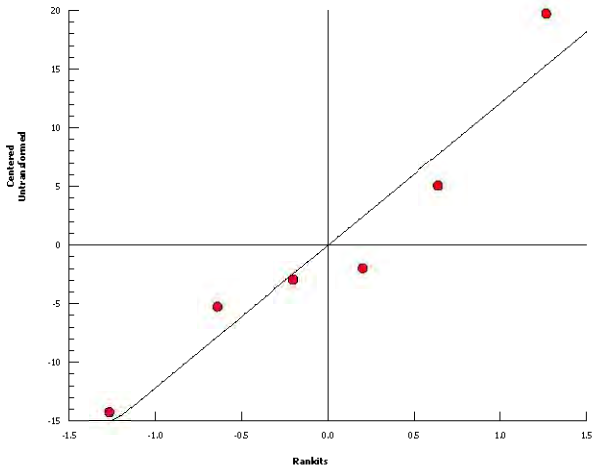
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	16.3	199	0.1154	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.938	0.43	0.6394	Normal Distribution

96h Cell Density Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	3	260	217	304	255	246	280	10.2	6.77%	0.0%
0.1		3	244	233	255	242	241	249	2.52	1.79%	6.27%

Graphics





OCSPP 850.4500 Algal Toxicity — **Bayer AG**

Analysis ID:	19-7069-2795	**Endpoint:**	96h Cell Density	**CETIS Version:**	CETISv1.8.7
Analyzed:	09 Feb-17 13:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-3415-0746	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	03 Aug-87	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Not specified
Ending Date:	07 Aug-87	**Species:**	Scenedesmus subspicatus	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C <> T	NA	NA	12.4%	Passes 96h cell density

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		Solvent Blank	0.0572	2.78	32.4	4	0.9571	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.6666667	0.6666667	1	0.00327	0.9571	Non-Significant Effect
Error	815.3333	203.8333	4			
Total	816		5			

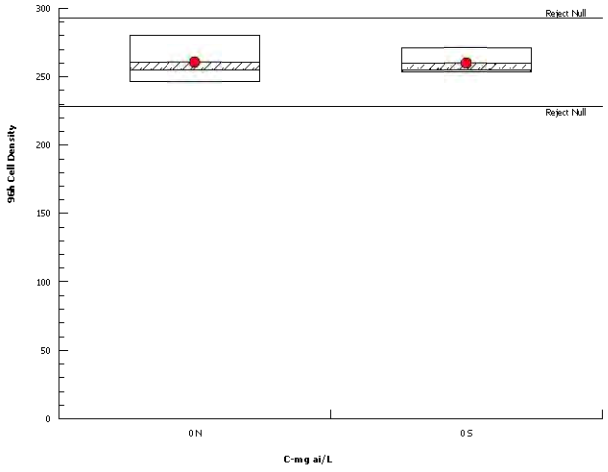
Distributional Tests

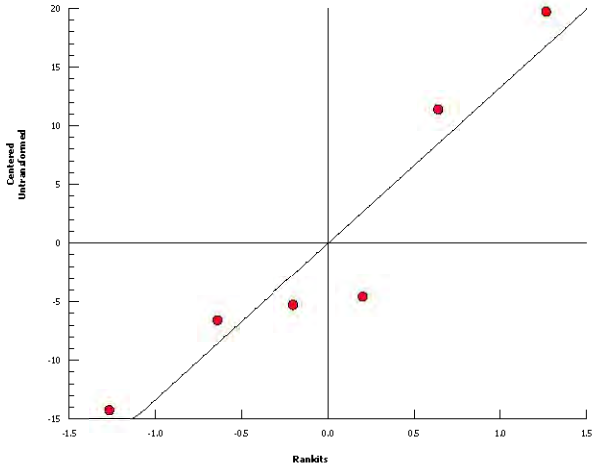
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	3.19	199	0.4775	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.892	0.43	0.3304	Normal Distribution

96h Cell Density Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	3	260	235	284	255	253	271	5.7	3.8%	0.0%
0	Negative Control	3	260	217	304	255	246	280	10.2	6.77%	-0.26%

Graphics





OCSPP 850.4500 Algal Toxicity — **Bayer AG**

Analysis ID:	01-1690-9110	**Endpoint:**	96h Growth Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	09 Feb-17 13:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-3415-0746	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	03 Aug-87	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Not specified
Ending Date:	07 Aug-87	**Species:**	Scenedesmus subspicatus	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	1.44%	Passes 96h growth rate

Equal Variance t Two-Sample Test

Control	vs	C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		0.1	2.12	2.13	0.020	4	0.0506	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0006	0.0006	1	4.5	0.1012	Non-Significant Effect
Error	0.0005333333	0.0001333333	4			
Total	0.001133333		5			

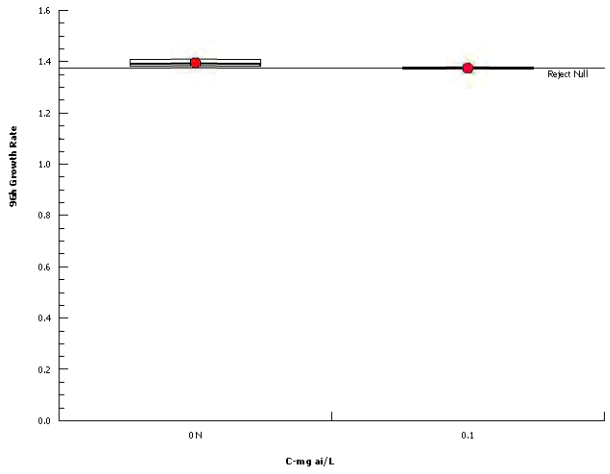
Distributional Tests

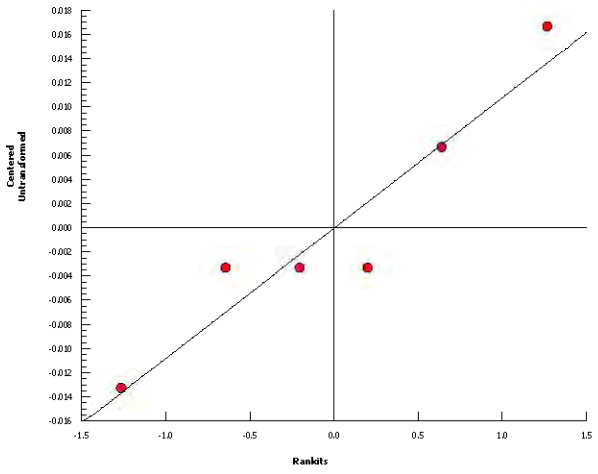
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	7	199	0.2500	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.915	0.43	0.4733	Normal Distribution

96h Growth Rate Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	3	1.39	1.36	1.43	1.39	1.38	1.41	0.00882	1.1%	0.0%
0.1		3	1.37	1.36	1.39	1.37	1.37	1.38	0.00333	0.42%	1.44%

Graphics





OCSPP 850.4500 Algal Toxicity — **Bayer AG**

Analysis ID:	16-6869-9592	**Endpoint:**	96h Growth Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	09 Feb-17 13:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-3415-0746	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	03 Aug-87	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Not specified
Ending Date:	07 Aug-87	**Species:**	Scenedesmus subspicatus	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C <> T	NA	NA	2.1%	Passes 96h growth rate

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		Solvent Blank	0.316	2.78	0.029	4	0.7676	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.666667E-05	1.666667E-05	1	0.1	0.7676	Non-Significant Effect
Error	0.0006666667	0.0001666667	4			
Total	0.0006833333		5			

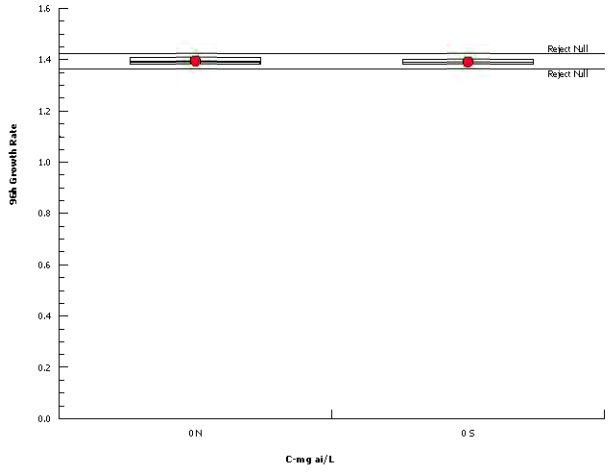
Distributional Tests

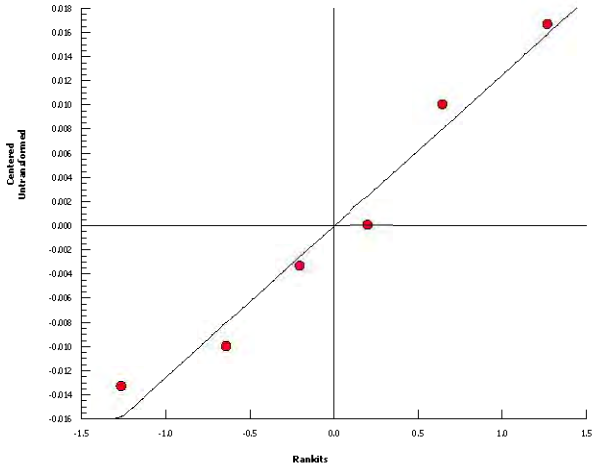
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.33	199	0.6000	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.952	0.43	0.7595	Normal Distribution

96h Growth Rate Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	3	1.39	1.37	1.41	1.39	1.38	1.4	0.00577	0.72%	0.0%
0	Negative Control	3	1.39	1.36	1.43	1.39	1.38	1.41	0.00882	1.1%	-0.24%

Graphics





OCSPP 850.4500 Algal Toxicity — **Bayer AG**

Batch ID:	16-3415-0746	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	03 Aug-87	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Not specified
Ending Date:	07 Aug-87	**Species:**	Scenedesmus subspicatus	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	
Sample ID:	17-5722-7886	**Code:**	49617843	**Client:**	CDM Smith - M. Wright
Sample Date:	03 Aug-87	**Material:**	Transfluthrin	**Project:**	
Receive Date:	07 Aug-87	**Source:**	Bayer CropScience		
Sample Age:	NA	**Station:**			

Batch Note: 129140 49617843
Sample Note: 129140 49617843

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-7447-2159	96h AUC	0	>0		15.1%		Equal Variance t Two-Sample Test
05-4300-0132	96h AUC	0.1	>0.1	NA	14.7%		Unequal Variance t Two-Sample Test
16-7848-5057	96h Cell Density	0.1	>0.1	NA	8.58%		Equal Variance t Two-Sample Test
19-7069-2795	96h Cell Density	0	>0		12.4%		Equal Variance t Two-Sample Test
01-1690-9110	96h Growth Rate	0.1	>0.1	NA	1.44%		Equal Variance t Two-Sample Test
16-6869-9592	96h Growth Rate	0	>0		2.1%		Equal Variance t Two-Sample Test

96h AUC Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	3	6240	5680	6790	6080	6490	129	223	3.58%	0.0%
0	Negative Control	3	6350	4970	7720	5880	6960	320	554	8.73%	-1.8%
0.1		3	6100	6030	6180	6070	6130	17.4	30.2	0.5%	2.12%

96h Cell Density Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	3	260	235	284	253	271	5.7	9.87	3.8%	0.0%
0	Negative Control	3	260	217	304	246	280	10.2	17.6	6.77%	-0.26%
0.1		3	244	233	255	241	249	2.52	4.36	1.79%	6.03%

96h Growth Rate Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	3	1.39	1.37	1.41	1.38	1.4	0.00577	0.01	0.72%	0.0%
0	Negative Control	3	1.39	1.36	1.43	1.38	1.41	0.00882	0.0153	1.1%	-0.24%
0.1		3	1.37	1.36	1.39	1.37	1.38	0.00333	0.00577	0.42%	1.2%

96h AUC Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3
0	Solvent Blank	6080	6130	6490
0	Negative Control	5880	6200	6960
0.1		6130	6110	6070

96h Cell Density Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3
0	Solvent Blank	255	253	271
0	Negative Control	246	255	280
0.1		249	241	242

96h Growth Rate Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3
0	Solvent Blank	1.39	1.38	1.4
0	Negative Control	1.38	1.39	1.41
0.1		1.38	1.37	1.37